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Ten years ago, Ivar Bang[1] succeeded in isolating from ox pancreas a nucleic acid which differed in a remarkable way from all known substances of this class. According to Bang the compound is characterized by the following properties.

(1) It contains a pentose group.

(2) It contains a glycerine group and yields glycerine phosphoric acid. The nucleic acid thus standing as a connecting link between the pentosans and the lecithins establishes a series of physiological chemical relations which can scarcely be found elsewhere in the science.

(3) It contains an amount of nitrogen and phosphorus relatively greater than is the case with other nucleic acids.

(4) It yields on hydrolysis an excessive amount of guanin but no trace of either adenin or thymin. On account of this striking difference from other nucleic acids, the substance has received the name of "guanylic acid."

This work which if true would constitute an unmistakable advance in the science has unfortunately been the subject of adverse criticism of a kind from which one would scarcely expect a recovery. Thus v. Fürth,[2] who has given the subject most careful attention, was able to find among the split products neither glycerine nor a reducing carbohydrate; he claims that the nucleic acid yields adenin as well as guanin and that there is no reason for making any distinction between guanylic acid and thymonucleic acid. In consideration of a number of objections all leading to the same conclusion one might reasonably suppose

[1] Bang: *Zeitschr. f. physiol. Chem.*, xxvi, p. 133; xxxi, p. 411.

[2] v. Fürth and Jerusalem: Hofmeister's *Beiträge*, x, p. 174.

that v. Fürth's work would finally dispose of the matter of guanylic acid. But Steudel[1] in a very recent communication, takes an entirely different view. He notes that the method of preparation employed by v. Fürth (proposed by Bang and Raaschou[2]) leads to a nucleic acid of the ordinary type, but that by following the original method of Bang it is possible to obtain from the ox pancreas a true guanylic acid, i. e., an organic compound containing phosphorus which yields guanin but not adenin. Unfortunately Steudel agrees with v. Fürth that guanylic acid contains no glycerine group.

It is the purpose of this communication to show that not only is a true guanylic acid obtainable from ox pancreas but that substances of this class are confined neither to a single animal species nor to a single gland.

THE PREPARATION OF THE NUCLEOPROTEIN OF PIG'S SPLEEN.

Fourteen kilos of carefully trimmed and finely ground fresh tissue were thoroughly mixed in portions with 21 liters of cold distilled water, heated quickly to boiling and the solution filtered off. It is immaterial whether the fluid be filtered hot or after cooling; in either case a fairly clear filtrate is obtained which by repeated filtration through the same filter becomes almost as clear as water but possesses a very pale reddish tinge. If the residue be pressed through linen a milky fluid results which we have not been able to render clearer, either by repeated filtration or by long continued centrifugation; it is therefore advisable to avoid disturbing the residue in any way and to use no greater amount of water for the extraction than that stated. This part of the process was exceedingly more satisfactory than was the case with beef pancreas (see below), where we were never able to obtain anything better than a highly opalescent filtrate, although the solution obtained from this gland is described by various writers as perfectly clear.

The neutral fluid when cool was treated with acetic acid up to 5 to 10 per mille and the precipitated gelatinous nucleoproteid was allowed to subside over night. This nucleoproteid is so

[1] Steudel: *Zeitschr. f. physiol. Chem.*, liii, p. 539.
[2] Bang and Raaschou: Hofmeister's *Beiträge*, iv, p. 175.

strikingly different in physical properties from the corresponding heavy flocculent substance of ox pancreas that we can scarcely believe the two substances identical. The cloudy fluid was partly decanted and the remainder sharply removed after centrifugation. The nucleoproteid which in this compact form resembles a preparation of starch paste, was freed from soluble impurities by alternate solution in a minimal amount of caustic soda and precipitation with the requisite amount of acetic acid. Suspended matter was removed as far as possible from each alkaline solution by long continued centrifugation, and in the same manner the precipitated nucleoproteid was sharply separated from the supernatant fluid. After this operation had been repeated several times a product was obtained which dissolved in a trace of alkali and on precipitation from the alkaline solution by acetic acid left a perfectly clear fluid. The exceedingly gelatinous nucleoproteid thus purified was dehydrated with alcohol and ether, but unless this is done with the greatest care one will finally obtain a brown sticky mass which is unsuitable for the work that follows. It is necessary to begin with dilute alcohol (50 per cent) and to replace this gradually until absolute alcohol is finally reached. The latter should be repeatedly used and the material allowed to stand for several days in well cooked flasks with frequent and violent agitation. We mention the difficulty of dehydrating this nucleoproteid because we encountered no such difficulty in dealing with the nucleoproteid of ox pancreas and we regard this very striking difference as sufficient ground for assuming that the two nucleoproteids are not identical. This is however entirely aside from the question of the identity of the two nucleic acids. From 14 kilos of moist tissue after the sacrifice of relatively large quantities of material in the interest of a pure product, we finally obtained 64 grams of a perfectly dry pale yellow powder.

THE PREPARATION OF GUANYLIC ACID FROM THE NUCLEOPROTEID OF PIG'S SPLEEN.

The nucleoproteid was treated in portions of 12 grams each with 150 cc. of 2 per cent caustic potash and heated for half an hour in a vessel submerged in boiling water. The red fluid was neutralized with acetic acid and while hot filtered from a small

quantity of perfectly black material; but even after standing 12 hours there was no deposition of guanylic acid although the solution was much more concentrated than that which Bang prepared from pancreas nucleoproteid. (We used only 150 cc. of 2 per cent caustic potash where Bang used 400 cc.) The perfectly clear yellow fluid was then made faintly acid with acetic acid and allowed to stand over night but there was still no deposition of guanylic acid. Several days later when we had concluded that this nucleoproteid yields no substance corresponding to pancreas guanylic acid and after we had practically abandoned the subject, a very small deposit was noticed in the fluid and on the addition of a few drops of acetic acid there was an immediate and copious precipitation of white flocculent material while practically all the coloring matter remained in solution. The precipitate was filtered off, dissolved in hot water and the solution filtered from a small amount of insoluble granular material. On cooling, the pale yellow fluid promptly deposited guanylic acid but on repeating the process the yield soon became noticeably smaller as the acetic acid was removed with the mother liquors. The addition of acetic acid to any of these filtrates causes an immediate precipitation of guanylic acid. This difference in behavior of spleen guanylic acid from pancreas guanylic acid might be explained by differences in the solvent power of the impurities in the two cases but spleen guanylic acid retains this property after purification to such an extent that we are inclined to the opinion that the two nucleic acids are not identical. The original neutral solution generally filters slowly but continuously and as a deposition of guanylic acid is not likely, the slowness of the filtration is without consequence. But it may happen that the neutral fluid cannot be filtered at all. In the one such case which we met, the fluid was markedly acidified with acetic acid, and cooled in ice water when clear and rapid filtration could be made leaving the guanylic acid on the filter. This dark brown residue was boiled with water and a product obtained which was easily filterable and which deposited guanylic acid on cooling. All specimens of what we considered the best products were collected and dissolved in hot water and the guanylic acid which was deposited on cooling the fluid was dried in the ordinary way, with alcohol and ether. From 52 grams of

nucleoproteid after severe losses in the mother liquors for the reason stated we finally obtained 1.58 gram of pure guanylic acid. The substance consists of a perfectly white dry powder soluble in hot water forming a transparent liquid which has an acid reaction to litmus. It responds neither to the biuret nor to Millon's reaction but contains phosphorus and exhibits general properties and reactions which closely accord with those which Bang describes for the guanylic acid of the pancreas.

THE PURIN BASES PRODUCED BY HYDROLYSIS OF THE GUANYLIC ACID OF PIG'S SPLEEN.

Owing to the misfortune of not knowing in the earlier part of our work that large quantities of guanylic acid may be recovered from mother liquors by the addition of acetic acid, the amount of material at our disposal was insufficient for an exhaustive examination such as we would otherwise have made and as we intend to make in the immediate future; so that we decided to devote all of our material to a final decision of the very important question, whether or not we are here dealing with a true guanylic acid.

A gram and a half of the material was heated for three hours with 25 cc. of 5 per cent sulphuric acid in a vessel submerged in boiling water. On standing over night the fluid deposited in profusion macroscopic needles of guanin sulphate. These were dissolved by warming and the solution was first neutralized and then treated with such an excess of ammonia that the fluid contained 2 per cent of the reagent. The product after digestion in the warm for an hour was allowed to cool and the precipitated guanin filtered off. After thoroughly washing in turn with 1 per cent ammonia and water the base was dissolved in 1 per cent caustic soda and again precipitated by the addition of acetic acid. The precipitate was filtered off, washed, dried and weighed. For the separation of guanin from small quantities of adenin there are two properties of the bases which can be used and can be thoroughly depended upon. First, guanin is almost insoluble in 2 per cent ammonia while adenin dissolves in this reagent with comparative ease. Second, both bases dissolve easily in dilute caustic soda but while guanin is quantitatively precipitated

from such a solution by acetic acid, adenin remains under these conditions completely in solution. It will be noticed that both of these methods were applied in turn to the case which we are describing. The original ammoniacal filtrate from guanin and the acetic acid fluid obtained in its purification were united and treated with silver nitrate and ammonia. The small silver precipitate was thoroughly washed, suspended in boiling water and decomposed with hydrochloric acid. The acid fluid was filtered from silver chloride, evaporated carefully just to dryness and the last traces of hydrochloric acid driven off by moistening with water and again carefully evaporating. The insignificant amount of residue was dissolved in water at 40° and treated with ammonia. A very small precipitate of guanin was formed which showed no inclination to dissolve in ammonia even after the addition of a great excess of the reagent. The fluid was filtered off and boiled until perfectly neutral to litmus. It will be observed that any adenin originally present must now be found in this fluid. Its volume was only 20 cc. yet a portion failed to give a distinct precipitate with silver nitrate and ammonia while in another portion picric acid did not even produce even an opalescence. *The guanylic acid of the spleen gives no trace of adenin.*

The main yield of guanin together with the small amount obtained from the mother liquors weighed 390 milligrams. It was dissolved in hot 5 per cent hydrochloric acid and decolorized with a small amount of animal charcoal. The solution on cooling deposited the characteristic centimeter—long feathery needles of guanin hydrochlorate. The salt was allowed to dry in the air and analyzed with the following results.

1. 0.1742 gram lost 0.0280 gram at 100° and required 7.06 cc. of standard sulphuric acid (1 cc. = 0.0077 gram of nitrogen).

2. 0.1921 gram lost 0.0309 gram at 100° and required 7.85 cc. of the same sulphuric acid.

	Required for $C_5H_5N_5O$. HCl. $2H_2O$:	Found: I.	II.
H_2O	16.11 per cent	16.07	16.08 per cent.
N	31.33 "	31.21	31.46 "

It may appear that our conclusion, viz: that we are here dealing with a guanylic acid, is based on an experiment with rather a small amount of material. In answer to such an objection we

would state that we should use no more if we were to repeat the work with an unlimited supply of material at our disposal. We are now engaged in an investigation of nucleic acids which involves a large number of just such analyses as that described and where the amount of material is of no consideration to us. Experience in these cases has taught us that the best quantitative results can be obtained by using no more nucleic acid than will produce 300 to 400 milligrams of the base sought.

ON THE DISTRIBUTION OF GUANYLIC ACID IN THE ORGANISM.

We are now occupied with the examination of a number of glands for substances of this type and have uniformly found substances whose physical properties correspond closely with those of guanylic acid. The pig's pancreas yields a perfectly clear aqueous extract from which acetic acid precipitates a flocculent nucleoproteid which closely resembles the nucleoproteid of ox pancreas and from which a guanylic acid can be prepared which cannot be distinguished by any apparent difference from ox pancreas guanylic acid. With ox pancreas we experienced considerable difficulty. The glands used were perfectly fresh and the method given by Bang closely followed but in spite of every effort we were unable to prepare any thing approaching a clear aqueous extract. However, the cloudy fluid gives a nucleoproteid which in turn yields a true guanylic acid.

Our results show conclusively that guanylic acid (or the guanylic acids[1]) are considerably more widely distributed than was formerly supposed and lend in great measure to the belief that these substances are common nuclear constituents. As all specimens of ordinary nucleic acid hitherto prepared have been found to yield adenin as well as guanin it seems certain that the glands which yield guanylic acid must also contain either "adenylic acid" or nucleic acids which produce both bases.

Since writing the above article our attention has been called to the work of Odenius[2] who prepared guanylic acid from the nucleoproteid of the mammary gland.

[1] It is possible that future investigations will demonstrate unmistakable differences among the nucleic acids of this class, thus establishing a series of guanylic acids.

[2] See Maly's *Jahresbericht*, xxxix, 1900.

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